Cancer Detection and Prevention Supplement 1:423–443 (1987)

Antitumor and Metastasis-Inhibitory Activities of Lentinan as an Immunomodulator: An Overview

Goro Chihara, DP
Junji Hamuro, DEng
Yukiko Y. Maeda, DP
Tsuyoshi Shiio, DAgr
Tetsuya Suga, MP
Nobuo Takasuka, MP
Takuma Sasaki, DP

National Cancer Center Research Institute (G.C., T.Su., N.T., T.S.), The Tokyo Metropolitan Institute of Medical Sciences (Y.Y.M.), Tokyo and Ajinomoto Central Laboratory, Yokohama (J.H., T.Sh.), Japan

ABSTRACT The antitumor and metastasis-inhibitory activities, mode of action, and clinical application of lentinan, a strictly purified β-1,6:β-1,3-glucan, are reviewed. Lentinan exerts a prominent antitumor effect and prevents chemical and viral oncogeneses. The antitumor action of lentinan is host-mediated. Compared to other well-known immunostimulants, such as bacille Calmette Guérin (BCG), *Corynebacterium parvum*, and lipopolysaccharide (LPS), lentinan appears to represent a unique class of immunopotentiator, a T cell-oriented adjuvant. Lentinan triggers the increased production of various kinds of bioactive serum factors associated with immunity and inflammation, such as IL-1, CSF, IL-3, vascular dilation inducer, and acute-phase protein inducer, by the direct impact of macrophages or indirectly via lentinan-stimulated T cells, which results in the induction of many immunobiological changes in the host. Augmented IL-1 production amplifies the maturation of immature effector cells to mature cells capable of responding to lymphokines such as IL-2 and T cell-replacing factors. Because of this mode of action, intact T cell compartments for antitumor activity of lentinan are required. Lentinan has little toxic side effects. Excellent results were obtained in a 4 year follow-up of the randomized control study of lentinan in phase III on patients with advanced and recurrent stomach and colorectal cancer.

Key words: lentinan, anticancer drug, oncogenesis prevention, immunostimulant

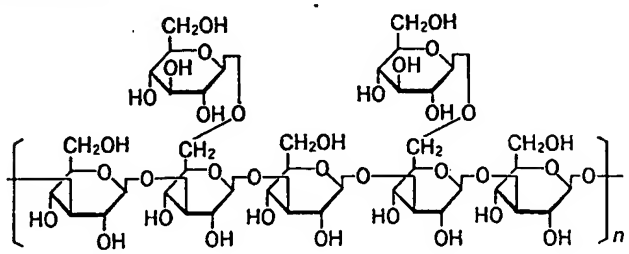
INTRODUCTION

In oriental medicine practiced in Asian countries, some kinds of fungi belonging to basidiomycetes have been used since olden times as folk remedies for cancer. Based on such a concept, we isolated a polysaccharide with marked antitumor activity from *Lentinus edodes* (Berk.) Sing., the most popular edible mushroom in Japan, and named it *lentinan* about 20 years ago [1,2]. Lentinan is a neutral polysaccharide, a fully purified β-1,3-D-glucan with β-

Address reprint requests to Dr. Goro Chihara, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan.

TABLE I. Structure and Physicochemical Properties of Lentinan

1. Primary structure



2. Higher structure
   Right-handed triple helical structure (by X-ray analysis)
   Lattice constant: hexagonal, a = b = 15 Å, c = 6 Å
3. Molecular formula (by elementary analysis)
   $(C_6H_{10}O_5)_n$: Culcd, C: 44.44%, H: 6.22%
   Found, C: 44.16%, H: 6.27%
   N, P, and S: Negative
4. Sugar component
   Glucose only (by gas chromatography)
5. Molecular weight
   Distribution in a range between $4 \times 10^5 - 8 \times 10^5$ daltons
   (by gel permeation chromatography and Laser Raman light scattering)
6. Physical constants
   Specific rotation: $[\alpha]_D^{20}$, 13.5 − 14.5° (in 2% NaOH), 19.5 − 21.5° (in 10% NaOH)
   UV spectra: No peak
   IR spectra: 890 cm^{-1} (β-glucose)
   Ultracentrifugation: One peak
   High voltage electrophoresis: One spot
   Solubility: Slightly soluble in water (0.1%)

1,6 branches having a triple helical structure, and its physical and chemical properties are strictly characterized [3,4] (Table I). This is the most important point of lentinan for immunopharmacological studies and clinical use.

Lentinan exerts a prominent antitumor activity in allogeneic, syngeneic, and autochthonous tumor-host systems, prevents chemical and viral oncogenesis [1,2,5–7], and increases host resistance to bacterial, viral, and parasitic infections [8,9]. Its immunopharmacological properties are well characterized as a T cell-oriented adjuvant in which macrophages play some part [10–18].

Lentinan caused little toxic side effects in in vivo application in animal models and human [19,20]. A four year follow-up of the phase III randomized control study of lentinan resulted in prolongation of life span of patients with advanced and recurrent stomach and colorectal cancer [21]. This review concerns the current status and perspectives on the antitumor and metastasis-inhibitory effects and mode of action of lentinan as an immunomodulator.

ANTITUMOR ACTIVITIES OF LENTINAN

The antitumor and metastasis-inhibitory effects of lentinan are summarized in Table II. It was initially found that lentinan caused complete regression of sarcoma 180 transplanted SC in Swiss albino or CD-1 mice at a dose of 1 mg/kg IP daily for 10 days [2].

TABLE II. Antitumor and Metastasis-Inhibitory Effects of Lentinan

Tumors*	Hosts	Dose of lentinan (1 mg/kg × days)	Route	Days of lentinan injection†	Tumor inhibition Ratio (%)‡	Complete regression of tumor
Allogeneic						
Sarcoma 180	CD-1/ICR	1 × 10	IP	1–10	100	10/10
	A/J	5 × 4	IP	1–4	96.5	9/10
	DBA/2N	5 × 4	IP	1–4	100	10/10
	SWM/Ms	1 × 10	IP	1–10	100	10/10
Ehrlich carcinoma	CD-1/ICR	1 × 10	IP	1–10	54.7	0/5
CCM adenocarcinoma	SWM/Ms	1 × 10	IP	1–10	65.3	0/10
Syngeneic						
A/Ph.MC.S1 fibrosarcoma	A/J	1 × 10	IP	1–10	100	18/18
DBA/2.MC.CS-1 fibrosarcoma	DBA/2N	1 × 10	IP	1–10	76.5	2/7
P-815 mastocytoma	DBA/2N	5 × 4	IV	8, 10, 15, 17	89.0	2/8
L-5178Y lymphoma	DBA/2N	10 × 3	IV	7, 14, 21	84.0	3/9
MM-46 carcinoma	C3H/HeN	5 × 2	IV	13, 15	100	9/9
Madison 109 carcinoma	BALB/c	25 × 2	IP	15, 18		8/22
Autochthonous						
MC-induced primary tumor§	DBA/2N	1 × 10	IP	1–10	80.5	2/5
Inhibition of metastasis						
DBA/2.MC.CS-1 fibrosarcoma	DBA/2N	1 × 10	IP	−11 to −1	94.2‖	
MH-134 hepatoma	C3H/HeN	1 × 14	IP	21–40	100¶	
Madison 109 carcinoma	BALB/c	25 × 2	IP	15, 18		10/14
Prevention of oncogenesis						Tumor occurrence
Methylcholanthrene-induced	SWM/Ms	1 × 10	IP	21–31		83 - 33%
Methylcholanthrene-induced	DBA/2N	1 × 10	IP	14–24		78 - 37%
Adenovirus type 12-induced	C3H/HeN	10 × 3	IP	14, 16, 18		79 - 40%

*All tumors were solid forms implanted s.c.
†Tumors were implanted on day 0.
‡Tumor inhibition ratio = (C − T)/C × 100. (C = average tumor weight of control mice: T = that of lentinan treated mice).
§Tumor grown to 5 mm diameter was day-0.
‖Colony inhibition in lung.
¶Survival after surgery.

Allogeneic Tumor-Host System

Several characteristics of the antitumor action of lentinan were observed. Its action was host-mediated without cytotoxicity against tumor cells when tested in cell culture systems. Lentinan generally had no antitumor effect in vivo against ascites tumor cells. Use of lentinan revealed an interesting phenomenon of optimal dose. A higher dose of lentinan injection showed considerably decreased antitumor effects. This phenomenon of optimal dose was also observed in various immune reactions induced by lentinan. Therefore, dose, frequency, timing, and route of lentinan administration are essential. There was also a marked difference in the antitumor effects of lentinan among various mouse strains.

The results of an antitumor assay of lentinan against sarcoma 180 in different inbred mice and their F1 hybrids are shown in Table III. Lentinan showed strong inhibitory effects on tumor growth in A/J, DBA/2, and SWM/Ms mice. When lentinan was used at the dose of 4 mg/kg IP daily for 5 days starting 1 day after SC tumor transplantation, almost all tumors underwent complete regression, and the inhibition ratios of tumor growth were 96.5, 100, and 100%, respectively. In contrast, C3H/He and C57BL/6 were low responder mice to lentinan treatment, and no complete regression was observed. BALB/c and CBA mice were moderately responsive. The susceptibility of tumors in the F1 hybrids to lentinan treatment seemed to be regulated by their parentage.

These results raise the question of why DBA/2, A/J, and SWM/Ms mice are high responders and C57BL/6 and C3H/He mice are low responders to the antitumor action of lentinan. The H-2 haplotypes, fur color genes of mouse strains, and allogenicity between tumor and host had no relationship to lentinan action. Therefore, certain host factors whose immune reactivity is regulated by lentinan may be the underlying cause of strain differences in lentinan action.

The relationship between the tumor susceptibility to lentinan treatment and the host immune response such as delayed-type hypersensitivity reaction (DTH), cytotoxic T lymphocytes (CTL), natural killer (NK) cells, and phagocytic macrophages, in inbred mouse strains

TABLE III. Effects of Lentinan Against Sarcoma 180 on Different Inbred Strains and Their F1 Hybrids

	Parent strains (H-2 haplotypes)						
	DBA/2 (dd)	A/J (aa)	BALB/c (dd)	CBA (kk)	C3H/He (kk)	C57BL/6 (bb)	SWM/Ms (?)
DBA/2							
IR (%)	100	98.7	96.6	90.7	64.3	87.5	
CR	5/5	4/6	2/6	3/6	1/6	0/8	
Response	High	High	High	High	Moderate	Moderate	
A/J							
IR		96.5	91.5	91.5	41.6	68.2	
CR		6/7	1/5	0/6	0/7	0/6	
Response		High	High	High	Low	Moderate	
BALB/c							
IR			80.6	75.7	36.6	43.4	
CR			3/6	2/7	0/7	0/6	
Response			Moderate	Moderate	Low	Low	
CBA							
IR				74.1	71.9	71.8	
CR				0/6	0/5	0/6	
Response				Moderate	Moderate	Moderate	
C3H/He							
IR					36.2	58.8	
CR					0/6	0/6	
Response					Low	Low	
C57BL/6							
IR						51.8	
CR						0/6	
Response						Low	
SWM/Ms							
IR							100
CR							6/6
Response							High

IR, Inhibition ratio of tumor growth; CR, complete regression of tumors. Antitumor assay: Dose of lentinan was 4 mg/kg IP daily for 5 days. Tumor transplantation was SC.

is summarized in Table IV. In general, multiple changes were detected in lentinan-treated animals, depending on the experimental system used. The DTH and/or CTL responses seemed to be the most important mechanism in these tumor-host systems, in which DBA/2, A/J and SWM/Ms mice were the most suitable strains.

Syngeneic Tumor-Host System

Because A/J, DBA/2, and SWM/Ms mice were suitable hosts for lentinan action, the effect of lentinan against syngeneic and autochthonous tumors was examined by use of these strains of mice. Lentinan injected IP at doses of 1 mg/kg daily for 10 days starting 1 day after tumor transplantation led to complete regression of methylcholanthrene(MC)-induced A/Ph.MC.S1 fibrosarcoma in A/Ph(A/J) syngeneic host. A/Ph mice in which this tumor had regressed were also able to reject a secondary challenge with the same tumor [5] (Fig. 1).

TABLE IV. Relationship Between the Antitumor Susceptibility to Lentinan Treatment and the Capability of Host Immune Factors

	ATS	DTH	CTL	NK	Mϕ
DBA/2	High		High		Low
A/J	High	High		Low	Low
SWM/Ms	High	High		Low	Low
BALB/c	Moderate			Low	
C3H/He	Low	Low		High	High
C57BL/6	Low			Moderate	High

ATS, antitumor susceptibility to lentinan treatment; DTH, delayed-type hypersensitivity reaction; CTL, cytotoxic T lymphocyte activity; NK, natural killer cell activity; Mϕ, macrophage phagocytic activity.

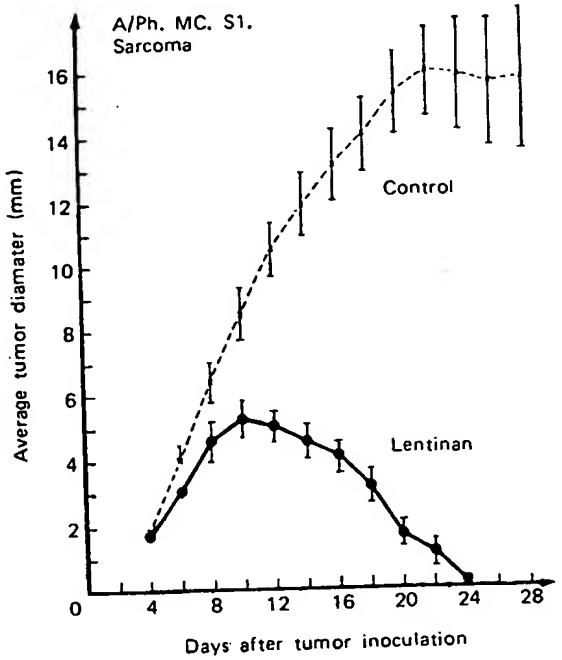


Fig. 1. Growth of syngeneic A/Ph.MC.S1 fibrosarcoma in control and lentinan-treated A/Ph mice. Both groups of mice (15 and 18, respectively) received 2.5×10^4 tumor cells ID and treatment with lentinan or control [5].

Lentinan showed a marked antitumor effect against the native and trypsinized DBA/2.MC.CS-1 fibosarcoma established in our laboratory for these experiments (Table V) [6]. As a trypsinized tumor should not be called a true syngeneic in the strict sense of the word, the native DBA/2.MC.CS-1 sarcoma was mainly used. When 0.1 ml of tumor cell suspension of native DBA/2.MC.CS-1 sarcoma was transplanted SC into syngeneic DBA/2 mice, the inhibition ratios of tumor growth induced by lentinan were 54.0 or 47.3% with a dose of 1 mg/kg × 10 or 10 mg/kg × 1, respectively. Lentinan was more effective against this tumor when DBA/2 mice were implanted with a smaller dose (1×10^4 cells) of tumor cells. The tumor inhibition ratio was 57.4%, and complete regression of tumor was observed in two out of five mice with 4 mg/kg × 4 by IP injection of lentinan. In the case of trypsinized DBA/2.MC.CS-1 sarcoma, the tumor inhibitory effect of lentinan was striking. With lentinan given in ten doses of 1 mg/kg each, tumor inhibition ratio was 76.5%, and tumors underwent complete regression in two out of seven mice.

Lentinan was also effective against various kinds of semisyngeneic tumors, such as P-815 mastocytoma and L-5178Y lymphoma in DBA/2 mice, virus-induced MM-46 and MM-102 carcinomas in C3H/He mice, and others (Table II). In these cases, timing of lentinan administration was very important. One hundred percent regression of MM-46 carcinoma was observed when 5 mg/kg of lentinan was injected twice, at 13 and 15 days after tumor transplantation.

Autochthonous Tumor-Host System

When DBA/2 mice were used, the IP injection of lentinan inhibited the growth of methylcholanthrene (MC)-induced autochthonous primary tumors [6] (Table VI). The tumors grew to 5 mm diameter within 15 weeks after MC treatment and were markedly inhibited by the IP injection of 1 mg/kg lentinan daily for 10 days starting at day 0 (grown to 5 mm diameter). The inhibition ratio of tumor was 80.5%, and the primary tumors underwent complete regression in two out of five mice. On the other hand, the tumor inhibitory effect of lentinan during 16 to 30 weeks after the MC inoculation was 50.4%, and no complete regression was observed, possibly because of a higher antigenicity of an earlier-occurring tumor than of a later-occurring one [22].

METASTASIS-INHIBITORY ACTIVITY OF LENTINAN

The correlation between spread of lymph node metastasis and prognosis after surgical resection of lung cancer was summarized by Watanabe and Suemasu [23] of the National

TABLE V. Effects of Lentinan on Growth of Native and Trypsinized DBA/2.MC.CS-1 Fibrosarcomas in DBA/2 Mice

DBA/2. MC.CS-1 fibrosarcoma	Treatment	Dose	D/T	Average tumor weight (gm)*	Tumor inhibition ratio (%)	No. of complete regressions
Native tumor†	Lentinan	1 mg/kg × 10	0/6	2.19	54.0	0/6
	Control		0/6	4.76		0/6
Native tumor†	Lentinan	10 mg/kg × 1	0/6	2.99	47.3	0/6
	Control		0/6	5.67		0/6
Native tumor‡	Lentinan	5 mg/kg × 4	0/5	0.84	57.4	2/5
	Control		0/6	1.97		0/6
Trypsinized tumor§	Lentinan	1 mg/kg × 10	0/7	0.47	76.5	2/7
	Control		0/7	2.00		0/7

†Tumor cell suspension 0.1 ml (1 gm of tumor tissue per 1 ml of saline); probably over 10^7 cells were transplanted SC.
‡Approximately 1×10^4 tumor cells were transplanted SC.
§Tumor cells 2.4×10^6 were transplanted SC.
*$P < 0.01$; Student t test compared to the control group.

TABLE VI. Antitumor Activity of Lentinan Against Methylcholanthrene-Induced Primary Tumors in DBA/2 and BALB/c Mice

Time of occurrence of MC-induced primary tumor	Lentinan treatment*	Dose	Average tumor weight (gm)	Tumor inhibition ratio (%)†	No. of complete regressions
DBA/2					
Within 15 weeks after MC treatment	Lentinan	1 mg/kg × 10	0.58	80.5	2/5
	Control		2.98		0/5
During 16 to 36 weeks after MC	Lentinan	1 mg/kg × 10	2.75	40.5	0/4
	Control		4.79		0/4
BALB/c					
Within 15 weeks after MC treatment	Lentinan	1 mg/kg × 10	0.21	77.7	1/6
	Control		0.94		0/9

*When every primary tumor had grown to 5 mm diameter, treatment of lentinan was started.
†$P < 0.01$ by Student t test compared to the control group.

TABLE VII. Spread of Lymph Node Metastases and Prognosis After Surgical Operation in Lung Cancer

Spread of lymph node metastasis	Case	Over-5-year survival cases/total operation cases	5-Year survival ratio (%)
No metastasis in lung	$n^0(-)$	88/172	51.1
Metastasis inner lung	$n^0(+)$	8/15	53.3
Metastasis: Hilus LN	n^1	32/95	33.7
Metastasis: Mediastinum	n^2	14/154	9.0
Metastasis: Other places	M1	0/33	0
Total		142/472	30.1

Data are from Watanabe and Suemasu [23] of the National Cancer Center Hospital, Tokyo.

Cancer Center Hospital, Tokyo (Table VII). In the cases of n^0 (−) lung cancer without any metastases in lung, the 5 year survival ratio of the patients was only 51.1% after surgical resection.

A fundamental concept in the clinical treatment of cancer metastasis is regression of a small number of autochthonous tumor cells scattering in the host. Because immunosuppressive anticancer drugs have a detrimental effect on cancer patients, the application of strong immunopotentiators such as lentinan should be suitable for adjuvant therapy after surgical resection.

Hematogenous Metastasis

Lentinan inhibited hematogenous pulmonary metastasis of syngeneic DBA/2.MC.CS-1 fibrosarcoma (Table VIII). After the IP injection of 1 mg/kg of lentinan daily for 10 days, 3×10^7 cells of this tumor were injected IV into the teil vein of DBA/2 mice. The lung metastases of this tumor were markedly inhibited by lentinan, and the metastasis inhibition ratio, calculated by the colony numbers of lung metastasis, was 94.2%.

Lymph Node Metastasis

Lentinan was also effective in lymph node metastases of MH-134 hepatoma. After SC inoculation of MH-134 hepatoma into syngeneic C3H/He mice, metastases occurred to lung, liver, heart, and other organs via lymph nodes. Lentinan prevented the recurrence of MH-134 hepatoma after tumor resection, and all mice survived when 1 mg/kg of lentinan was injected IP daily for 10 days after surgical resection (Fig. 2).

TABLE VIII. Inhibition of Hematogenous Pulmonary Metastases of Syngeneic DBA/2.MC.CS-1 Fibrosarcomas by Lentinan

No. of tumor cells*	Lentinan injections (1 mg/kg × 10)†	Average colony No. of lung metastasis	Metastasis inhibitory ratio (%)
1×10^5	−	7.0	—
1×10^5	+	0.6	91.4
3×10^5	−	27.8	—
3×10^5	+	1.6	94.2
1×10^6	−	34.0	—
1×10^6	+	5.3	84.4

*IV injection from teil vein.
†IP injection from 10 days before tumor implantation.

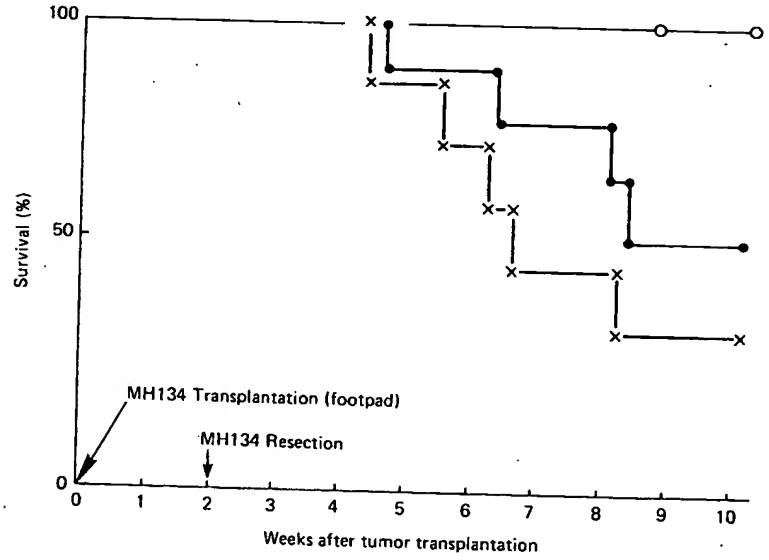


Fig. 2. Inhibition of postoperative MH-134 hepatoma metastasis by lentinan administration. ×, Control (surgery only); ●, surgery plus lentinan 1 mg/kg/day IP × 14 from 1 to 14 days after tumor transplantation; ○, surgery plus lentinan 1 mg/kg/day IP × 14 from 15 to 28 days after tumor transplantation; MH-134 transplantation, foodpad SC; MH-134 resection, 2 weeks after tumor transplantation.

Similar results using Madison 109 lung carcinoma and lentinan were obtained by Rose et al. [24]. They reported that lentinan prevented death from metastases after surgical resection of this tumor. The effects of surgery alone and surgery followed by an injection of 25 mg/kg of lentinan compared with untreated control are demonstrated in Figure 3.

PREVENTION OF ONCOGENESIS BY LENTINAN

Lentinan prevented chemical and viral oncogenesis [6,7]. Hamada [7] reported that lentinan inhibited adenovirus-induced oncogenesis. When newborn C3H/He mice were infected with 10^7 $TCID_{50}$ of adenovirus type 12, the tumor incidence was about 80% at day 80 after the infection, whereas the tumor incidence in mice given triple injections of 10 mg/kg of lentinan at 14, 16, and 18 days after the infection was about 40% (Fig. 4). Prevention of oncogenesis may be considered as an experimental method for inhibition of metastasis because of the control of small amounts of autochthonous tumor cells in the host.

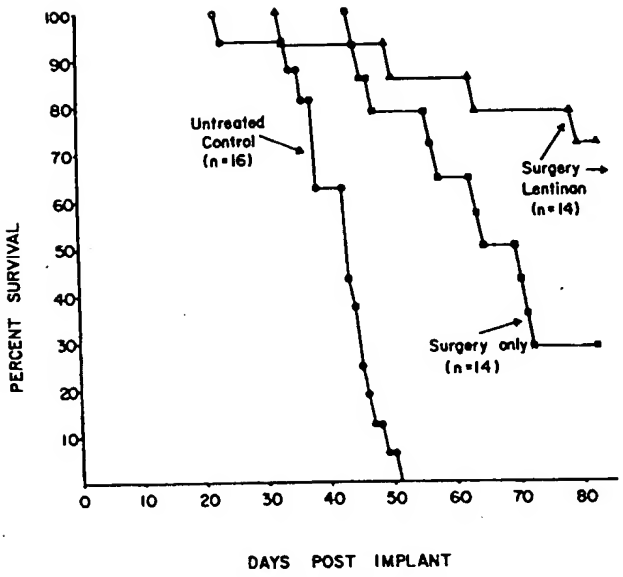


Fig. 3. Inhibition of postoperative syngeneic Madison 109 lung carcinoma metastases in BALB/c mice by lentinan injection. Data from untreated controls, surgery only, and surgery plus lentinan 25 mg/kg/day IP × 2 from 1 and 4 days after tumor resection are compared. Madison 109 implantation, foodpad SC; Madison 109 resection, 13 or 14 days after tumor implantation. Data are from Rose et al [24].

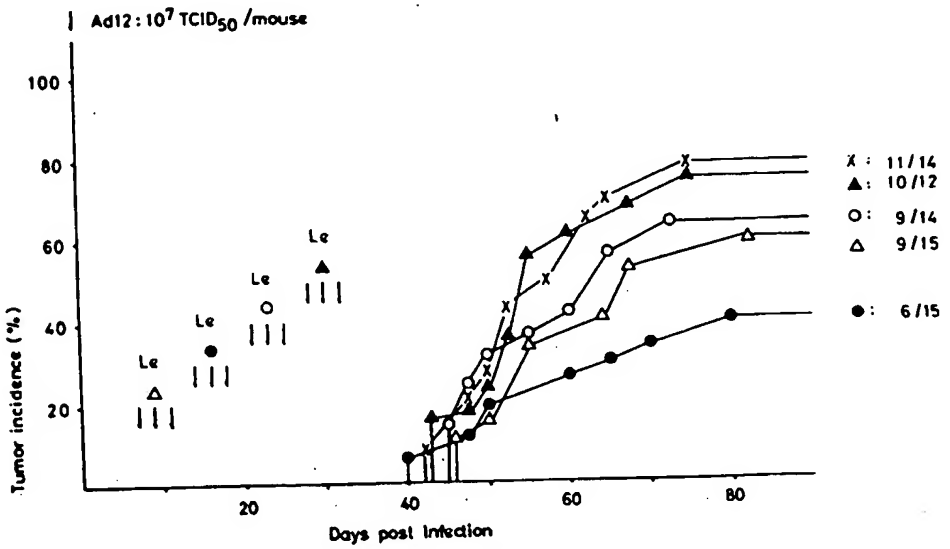


Fig. 4. Inhibition of the development of adenovirus type 12-induced tumor by lentinan. Newborn C3H/He mice were infected with 1×10^7 $TCID_{50}$ of adenovirus type 12. Lentinan treatment (10 mg/kg) was given on days 7, 9, and 11 (△); 14, 16, and 18 (●); 21, 23, and 25 (○); and 28, 30, and 32 (▲) postinfection, respectively. Control mice were infected but untreated (×). Data are from Hamada [7].

We found that lentinan suppressed chemical carcinogenesis [6]. When 1 mg/kg of lentinan was injected IP daily for 10 days into DBA/2 mice from 2 weeks after MC treatment, the tumor occurrence ratio was about 30% after week 40 compared to about 80% in untreated control mice (Fig. 5).

In another experiment, timing of lentinan administration for prevention of chemical carcinogenesis was examined using the high responder SWM/Ms mice. Tumor incidence was strikingly suppressed in the mice that were given lentinan 3 weeks after MC inoculation. However, lentinan given 6 weeks after MC treatment was less effective (Fig. 6). This suggests a possible effectiveness of lentinan on micrometastasis after surgical resection in cancer patients, because the small number of autochthonous tumor cells that may have occurred in the host within 3 weeks after MC inoculation had regressed through immunopotentiation by lentinan.

MODE OF ACTION OF LENTINAN AS A T-CELL ADJUVANT

Lentinan has no direct cytotoxicity against tumor cells, and its antitumor action is host-mediated. The immunological activities of lentinan are listed in Table IX. The antitumor activity of lentinan was absent in neonatally thymectomized mice [11] and was decreased by the administration of antilymphocyte serum [25]. These results suggest that the antitumor action of lentinan requires an immunocompetent T-cell compartment and that the activity is mediated through thymus-dependent immune mechanisms. Antitumor effects of lentinan were also inhibited by pretreatment with the antimacrophage agents carrageenan and silica. Thus, lentinan is a T cell-oriented adjuvant in which macrophages play some parts. Among the well-known immunostimulants, such as BCG, *C. parvum*, and LPS, lentinan appears to represent an unique class of immune adjuvant.

It is, however, not clear how lentinan affects the host at a stage before the induction of many immunological changes. It is suggested that the biological activities of lentinan may depend on the presence of certain substances or cells in the host that interact with lentinan.

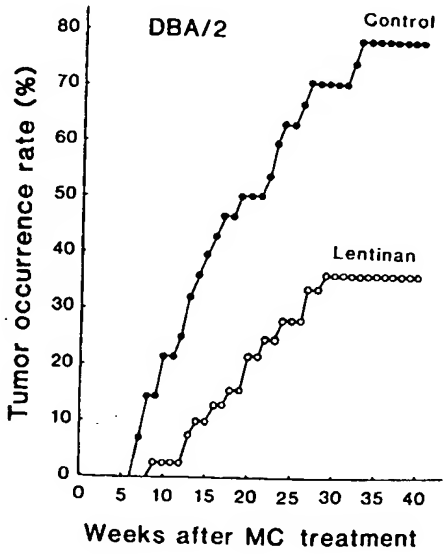


Fig. 5. Preventive effect of lentinan on 3-methylcholanthrene-induced carcinogenesis in DBA/2 mice. Data from controls (no lentinan treatment) and mice receiving lentinan injections (1 mg/kg IP daily for 10 days) started 2 weeks after MC treatment are shown.

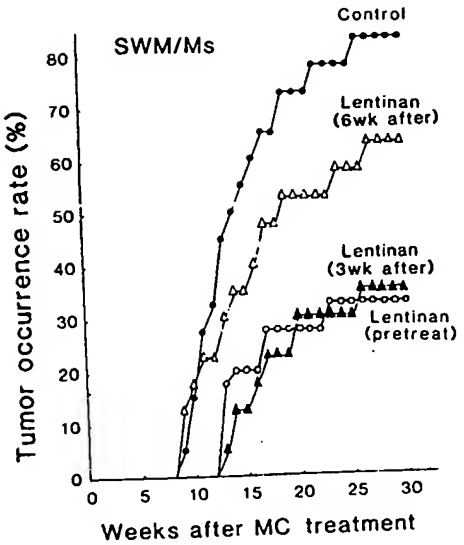


Fig. 6. Significance of timing of lentinan injections in prevention of 3-methylcholanthrene-induced carcinogenesis. ●, Control (no lentinan treatment); ○, lentinan injection begun 10 days before MC inoculation; ▲, lentinan injections begun 3 weeks after MC inoculation; △, lentinan injections begun 6 weeks after MC inoculation. Dose of lentinan was 1 mg/kg IP daily for 10 days.

Appearance of Serum Bioactive Factors Soon After Lentinan Injection

We found a transitory increase in various serum protein components and bioactive serum factors soon after lentinan administration (Table X). These are IL-1 production-inducing factor (IL1-IF), IL-3, and colony-stimulating factors (CSF) [26], vascular dilatation and hemorrhage-inducing factor (VDHIF) [27], and acute-phase transport protein-inducing factor (APPIF) [28]. These factors were produced by mainly accessary macrophages or lentinan-stimulated T cells, and the increase in these factors resulted in the activation of lymphocytes, hepatocytes, and probably mastocytes and the development of many biological reactions in the host. Complement components also increased in the lentinan-treated mice.

Acute-Phase Transport Protein-Inducing Factor

Several protein components increased markedly in the mouse serum 4 to 7 days after lentinan injection [29]. These were identified by the two-dimensional electrophoretic method as acute-phase transport proteins such as haptoglobin, hemopexin, and ceruloplasmin [30].

We recently found a factor that regulates the increase of acute-phase transport proteins in the serum (L10-6h serum) that was obtained from mice 6 hours after an injection of 10 mg/kg of lentinan [28]. The acute-phase transport proteins were markedly increased 4 days after the IV injection of L10-6h serum, and this increase was inhibited by pretreatment with the antimacrophage agents carrageenan and anti-Ia antiserum of donor mice (Fig. 7). Therefore, APPIF appears to be a product of macrophages, and it may stimulate hepatocytes to prepare the acute-phase proteins.

Vascular Dilation and Hemorrhage-Inducing Factor

L10-6h serum was found to induce vascular dilatation and hemorrhage [27]. This factor also appeared to be a product of macrophages, because the injection of carrageenan before lentinan treatment inhibited its production. This reaction, however, was thymus-dependent, because L10-6h serum did not induce the vascular dilatation reaction in nude mice. L10-6h

TABLE IX. Immunological Activities of Lentinan

1. T cell participation	
Neonatal thymectomy	Abolished antitumor effect
Antilymphocyte serum	Decreased antitumor effect
Helper T cell in vitro	No observed effect
Helper T cell in vivo	Activation or restoration
Cytotoxic T cell in vitro	Augmentation of IL-2 responsibility
Cytotoxic T cell in vivo	Increased responsibility to IL-2
Suppressor T cells	No induction
Migration inhibitory factor-producing T cells	Activation
IL-3	Increased production
T cell-derived CSF	Increased production
2. Natural killer cell participation	
NK cells in vitro	No effect
NK cells in vivo	Activation in C3H/He, but not BALB/c mice
Augmented NK activity by poly I:C or IL-2 in vitro	More activation when used in lentinan-treated mouse spleen cells
3. Macrophage participation	
Antimacrophage agent	Decreased tumor suppressive effect by carrageenan and silica
Macrophage: Phagocytic in vitro	No effect
Macrophage: Phagocytic in vivo	Very weak effect
Macrophage: Cytotoxic in vitro	Not observed
Macrophage: Cytotoxic in vivo	Activation
Macrophage: Suppressive in vivo	Decreased prostaglandin E release from macrophages
IL-1	Increased production in vitro and in vivo
4. Antibody formation	
Antibody for SRBC	Increased production with T cells
Antibody-dependent cell-mediated cytotoxicity	Activation
5. Cellular reactions	
Delayed hypersensitivity in vivo	Stimulation or restoration
Local cellular reaction	Increase around tumor
Granuloma formation	Increase around Shistosoma
6. Complement participation	
Alternative pathway	Activation
C3 splitting activity	Activation
C3 absolute value	Increased production
Total complement value	Increased production
Classical pathway	Activation

serum may contain a histamine-producing cell stimulating factor. Interestingly, there is a close correlation between antitumor activity and vascular dilatation-stimulating activity of lentinan.

IL-1 Production-Inducing Factor, Colony-Stimulating Factor, and IL-3

Lentinan triggers the increased production of IL-1 by a direct impact on macrophages or indirectly via colony-stimulating factor (CSF) from lentinan-stimulated T cells.

After lentinan injection two peaks of increased CSF were observed (Fig. 8). Three hours after lentinan injection the appearance of CSF in the serum was found to be generated from alveolar macrophages that were stimulated by lentinan. However, the CSF that appeared several days after lentinan injection was found to have originated from T lymphocytes, because this increase was not present in nude mice. Similarly, IL-1 production-inducing activity in the

TABLE X. Increase of Protein Components and Bioactive Factors in Mouse Serum After Lentinan Administration

Activities (peak, 2–24 hours after)	Components (peak, 3–7 days after)
IL-1 production-inducing factor	Interleukin-1
Interleukin-3	Colony-stimulating factor (T cell-derived)
Colony-stimulating factor (directly from macrophages)	Haptoglobin
Acute-phase protein-inducing factor	Hemopexin
Vascular dilatation and hemorrhage-inducing factor	Ceruloplasmin
Lysozyme activity	Serum amyloid P
	Complement C3
	Complement C5
	Complement factor B

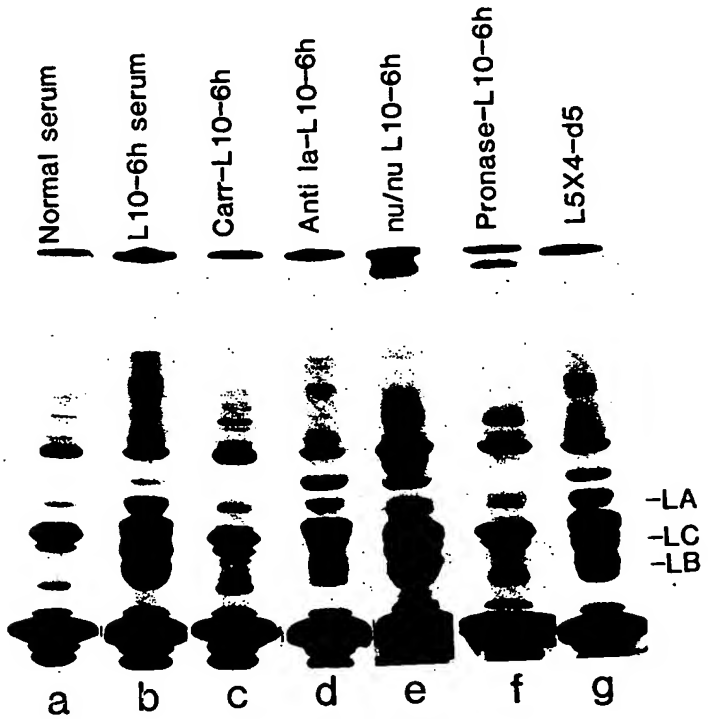


Fig. 7. Polyacrylamide gel electrophoresis patterns of serum samples obtained from mice 4 days after the injection of 0.2 ml per mouse of various serum samples: **a:** Normal CD-1 mouse serum; **b:** L10-6h serum; serum obtained from CD-1 mice 6 hours after IP injection of 10 mg/kg of lentinan; **c:** Carr-L10-6h; L10-6h serum obtained from the mice that received 100 mg/kg of carrageenan 24 hours before lentinan injection; **d:** Anti-Ia L10-6h; L10-6h serum obtained from mice that received 0.1 ml of anti-Ia antiserum 5 to 10 minutes before lentinan injection; **e:** nu/nu L10-6h; L10-6h serum obtained from athymic nu/nu CD-1 mice; **f:** Pronase-L10-6h; pronase E-treated L10-6h serum; **g:** L5×4-d5: Serum sample obtained from CD-1 mice on day 4 after the final injection of 5 mg/kg of lentinan daily for 4 days.

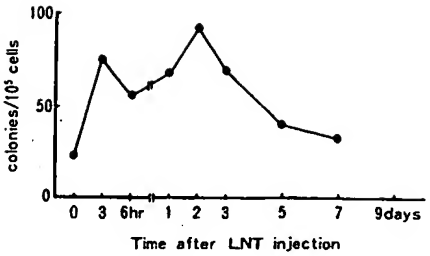


Fig. 8. Colony-stimulating activity (CSA) in the serum of lentinan-injected DBA/2 mice. Serum was harvested at 0, 3, and 6 hours and 1, 2, 3, 5, and 7 days after an IV injection of 10 mg/kg of lentinan. CSA assay was a soft-gel system; 10^5 C57BL/6 bone marrow cells per dish were cultured for 7 days.

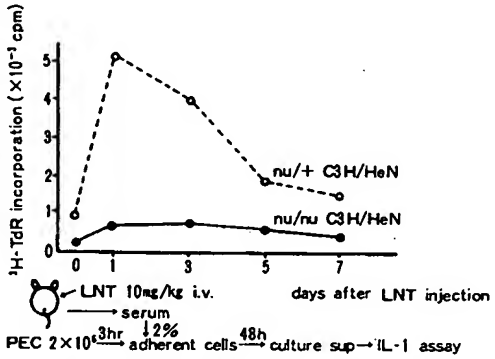


Fig. 9. IL-1 production-inducing activity in the serum of lentinan-injected mice.

	ILI (⊿cpm)	CSA (colonies/10^5 cells)
normal serum (2%)	−10	159
LNT serum (2%)	245	821
medium	23	31

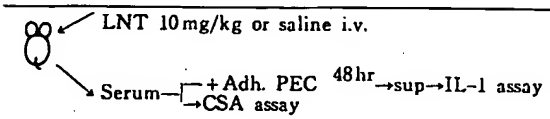


Fig. 10. Correlation between IL-1 producing activity and colony-stimulating activity induced by lentinan. IL-1 (Δcpm) was determined by tritium incorporation into thymocytes. Δcpm = (+ serum or medium) − (− serum or medium). CSA increased in early phase augments IL-1 production by macrophages.

serum of lentinan-injected mice was also absent in nude mice (Fig. 9). Thus, T cells seem to play a relevant role in the generation of IL-1 production-inducing factor and of CSF. There is a correlation between IL-1 producing activity and CSF activity induced by lentinan (Fig. 10). Increased CSF in the early phase augments IL-1 production of macrophages. The augmented effect was also demonstrated by use of chromatographically purified CSF [26].

Direct Production of IL-1 From Monocytes and Macrophages

On the other hand, augmented production of IL-1 was observed when human monocytes [31], murine peritoneal cells, or macrophage cell line P388D1 were cocultivated with lentinan in vitro. The peritoneal adherent cells obtained from the lentinan-treated mice produced increased amounts of IL-1 in cultured supernatant (Fig. 11).

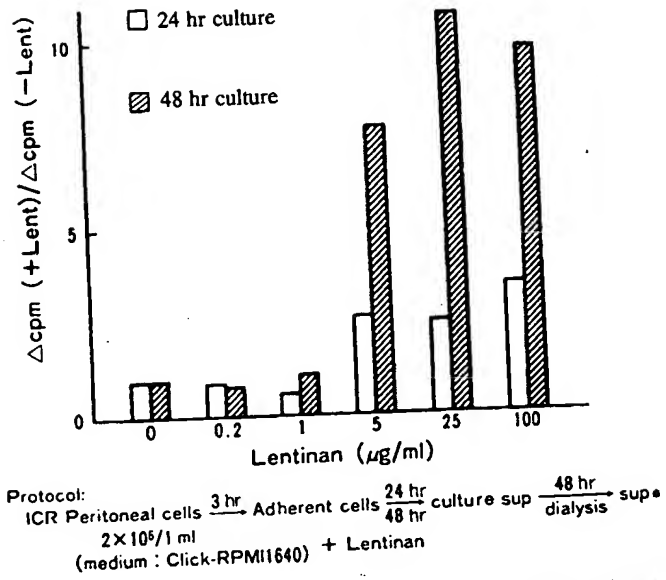


Fig. 11. Direct effect of lentinan on augmented production of IL-1 from murine peritoneal cells.

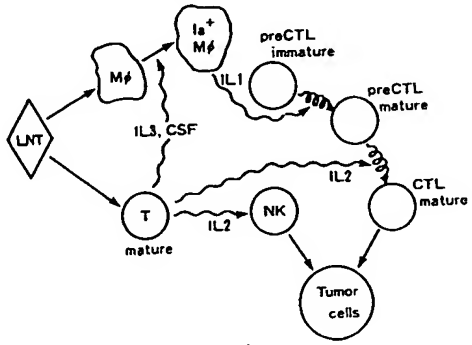


Fig. 12. Cytokine(s) production and induction of effector cells by lentinan. Straight lines show direct action, wavy lines show factors, and coiled lines show differentiation.

Cytotoxic T Lymphocytes and Lentinan

In view of the fact that IL-1 is capable of differentiating thymic premature T cells into immunocompetent mature T cells, the findings presented here suggest that lentinan promotes the differentiation of premature T cells into immunocompetent mature T cells. The augmented cytokine production, induced by lentinan, apparently stimulates generation of effector cells against tumor cells (Fig. 12). This mode of action suggests the requirement of an intact T cell compartment for the antitumor activity of lentinan. To evaluate the validity of the explanation, the generation of allokiller cells from thymocytes was tested. Thymocytes, harvested from BALB/c mice and treated with lentinan, induced augmented generation of allokiller cells in the presence of IL-2 (Fig. 13). This demonstrates that thymocytes of lentinan-treated mice had an increased reactivity to IL-2.

In a syngeneic tumor-bearing system such as P815-DBA/2, the generation of allokiller cells from thymocytes is considerably suppressed even in the presence of IL-2. After injection of lentinan, however, the generation of allokiller cells was restored to the level of control mice. The generation of allokiller cells from splenocytes was also restored by lentinan injection

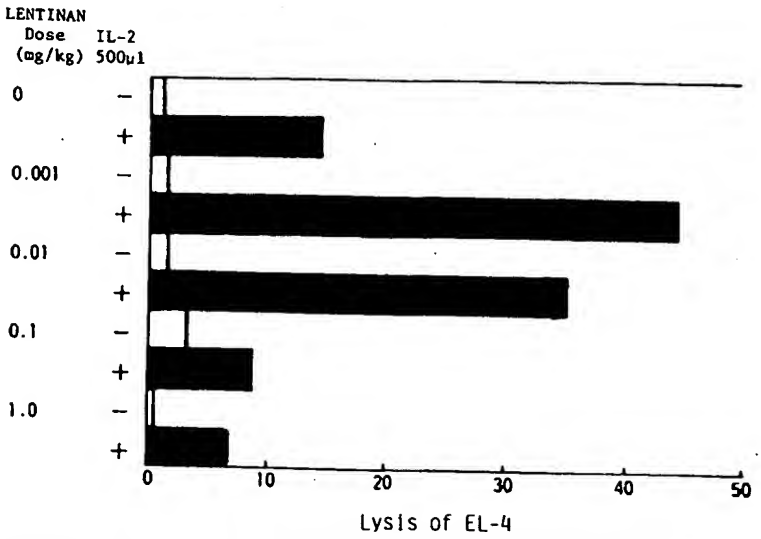
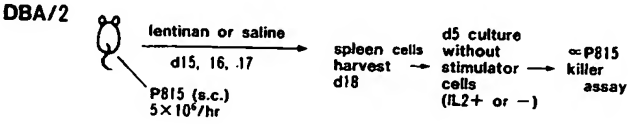


Fig. 13. Lentinan augments sensitivity of thymocytes to IL-2. Augmented allokiller T cell induction by lentinan from thymocytes in the presence of IL-2. Mixed lymphocyte culture: BALB/c C57BL/6; E:T = 3:1.



lentinan dose (mg/kg×3)	P815-bearing mice lysis of P815 (%) IL2−	IL2+
0	0	6
0.1	4	19
1	1	22
10	2	27

Fig. 14. Augmented allokiller T cell generation by lentinan in a P-815 syngeneic tumor-bearing DBA/2 mouse system. Effector:Target = 50:1 (culture base). Killer assay, 3 hours, ^{51}Cr release.

from 0 to 40%. These results may explain the increase in the formation of mature T cells reactive to IL-2. Furthermore, spleen cells harvested from syngeneic tumor-bearing mice that had received triple IP injections of lentinan were able to generate significant levels of antisyngeneic tumor killer cells in the presence of IL-2 (Fig. 14). This suggests that lentinan may restore decreased immune reactivity in cancer and AIDS by the combination therapy with recombinant IL-2.

Delayed Hypersensitivity Reaction and Lentinan

Delayed-type hypersensitivity (DTH) reaction is an important mechanism of lentinan action. Lentinan restored the decreased DTH reaction in tumor-bearing hosts. Furthermore, DTH reaction was markedly enhanced by the administration of lentinan, when the syngeneic A/Ph.MC.S1 fibrosarcoma had completely regressed [32,33]. This augmenting effect produced by lentinan injection may be explained also by the augmenting effect of lentinan on the

reactivity of macrophages to macrophage-activating factor (MAF) as demonstrated in tumor antigen-directed DTH [18].

Natural Killer Cells and Lentinan

Concerning the other important nonspecific effector, natural killer (NK) cells, it was found that lentinan could augment NK activity in spleen cells and peritoneal exudate cells when administered into NK high responder C3H/He mice, contrary to its administration into BALB/c mice. However, lentinan did not activate NK cells when incubated in vitro, as poly I:C and zymosan do [18].

Augmented NK cell generation was observed when spleen cells obtained from lentinan-treated mice were cultured with poly I:C or IL-2 (Fig. 15). Because lentinan does not activate NK cells in vitro, it seems likely that in vivo application of lentinan results in the augmentation of NK cell reactivity to IL-2.

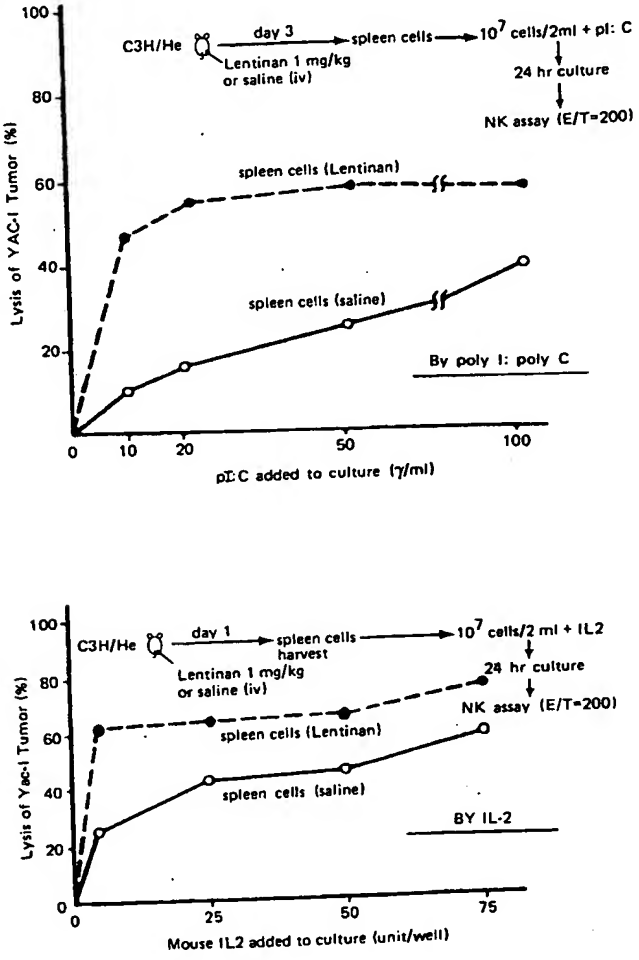


Fig. 15. Synergistic augmentation of natural killer cells by lentinan with poly I:C and with IL-2 in vitro. Natural killer activity was assayed using ^{51}Cr-labeled YAC-1 cells as target cells in a 4 hour ^{51}Cr-release assay at a ratio of effector:target = 200:1.

Complement and Nonspecific Cytotoxicity of Macrophages

Lentinan not only augments antigen-specific cellular immune responses by increased responsibility to IL-2 and cytokines, but is also capable of triggering nonspecific immune responses against neoplastic cells. Lentinan activated the alternative pathway of complement system [34,35]. Lentinan splits C3 into C3a and C3b in vitro, resulting in augmented generation of nonspecific cytotoxicity of macrophages. Peritoneal exudate cells obtained from lentinan-treated mice showed considerable nonspecific cytotoxicity against sarcoma 180, Ehrlich carcinoma, and L5178Y lymphoma [36]. The cytotoxicity of peritoneal macrophages harvested from lentinan-treated CBA mice was over 80% lysis against P-815 mastocytoma, while the lysis with control mice was only 2.5% [18]. Lentinan, however, did not have any cytotoxicity in in vitro experiments.

Lentinan, nevertheless, did not enhance the phagocytic activity of macrophages in vitro and in vivo as assessed by a carbon clearance test [11]. Therefore, lentinan seems to be distinct from most immune stimulants, so-called RES stimulants.

Conclusion Regarding Mechanism of Action

Lentinan appears to be a unique immunological adjuvant with nontoxic side effects from in vivo application. In light of the various described immunological characteristics of lentinan, the possible mode of action of lentinan is tentatively suggested although there are still many points to be elucidated (Fig. 16).

CLINICAL APPLICATION AND POSSIBLE FUTURE TRENDS

Lentinan was found to have a distinct antitumor and metastasis-inhibiting effect in allogeneic, syngeneic, and autochthonous hosts, and its unique mode of action has been evaluated. Many acute, semiacute, and chronic toxicological studies have been completed in animal models, and the LD_{50} is over 2,500 mg/kg IP and 250–500 mg/kg IV in mice and rats. Therefore, lentinan is worthy of consideration for effective therapy of cancer patients.

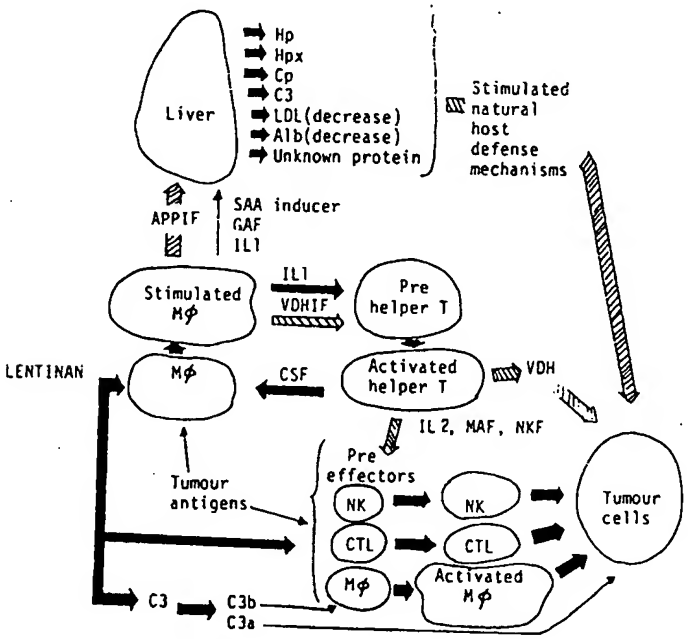


Fig. 16. Possible mode of action of lentinan.

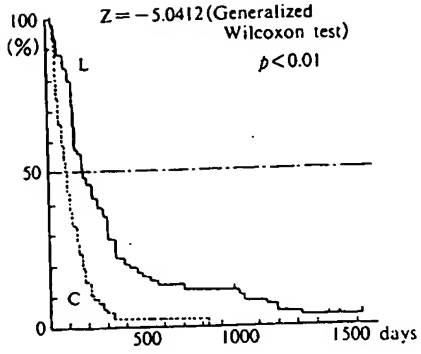


Fig. 17. Clinical application of lentinan on survival curves from patients with advanced and recurrent gastric cancer—4 year follow-up results of a randomized control study of lentinan in phase III. L, lentinan-treated group, 2 mg/person/week of lentinan plus 400–1200 mg/person/day of Tegaful; C, control group, 400–1200 mg/kg of Tegaful alone. Drawn by Kaplan-Maier's method and examined by generalized Wilcoxon's test. Control group, 68 cases, 50% survival 92 days. Lentinan group, 77 cases, 50% survival 173 days. Data are from Taguchi et al [21].

Taguchi and cooperative clinical study groups investigating lentinan have carried out phase I, II, and III studies of randomized control clinical trials of lentinan [20]. Recent results of a 4 year follow-up phase III randomized control study of lentinan patients with advanced and recurrent stomach and colorectal cancer revealed that the combination therapy of lentinan with Tegaful led to prolongation of their lifespan (Fig. 17) [21]. In the case of stomach cancer, the percent survival ratios of the lentinan-treated patients were 24.32, 12.97, 9.51, and 3.81%, while those of the control group (Tegaful only) were only 3.70, 3.70, 0, and 0% in 1, 2, 3, and 4 year results, respectively.

These results suggest that the use of lentinan may increase the survival time and cause the complete cure of micrometastases after surgical resection in cancer patients. Human cancer is a disease with great diversities comparable to all infectious diseases. Tumor-host relationships may differ depending on the stage of tumor growth and on the course of treatments. Therefore, the use of lentinan for human cancer should be based on strict injection times and dose schedules in compliance with the immunological and biological changes that were observed in lentinan-treated animals. The effect of lentinan in the treatment of human cancer is increased when used in conjunction with other therapies, especially after surgical resection. An important task for clinical immunologists is to determine parameters for tumor-host relationships which serve as indicators for protocols on the use of immunopotentiators such as lentinan.

REFERENCES

1. Chihara G, Maeda YY, Hamuro J, Sasaki T, Fukuoka F. Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. Nature 1969; 222:687–8.
2. Chihara G, Hamuro J, Maeda YY, Arai Y, Fukuoka F. Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing., an edible mushroom. Cancer Res 1970; 30:2776–81.
3. Sasaki T, Takasuka N. Further study of the structure of lentinan, an antitumor polysaccharide from *Lentinus edodes*. Carbohydrate Res 1976; 47:99–104.
4. Bluhm TL, Sarco A. The triple helical structure of lentinan, β-(1-3)-D-glucan. Can J Chem 1977; 55:293–9.
5. Zákány J, Chihara G, Fachet J. Effect of lentinan on tumor growth in murine allogeneic and syngeneic hosts. Int J Cancer 1980; 25:371–6.
6. Suga T, Shiio T, Maeda YY, Chihara G. Antitumor activity of lentinan in murine syngeneic and autochthonous hosts and its suppressive effect on 3-methylcholanthrene induced carcinogenesis. Cancer Res 1984; 44:5132–7.

7. Hamada C. Inhibition effect of lentinan on the tumorigenesis of adenovirus type 12 in mice. In Aoki T, Urushizaki I, Tsubura E, eds: Manipulation of host defence mechanisms. Amsterdam: Excerpta Medica, 1981; 76–87.
8. Kanai K, Kondo E, Jacques PJ, Chihara G. Immunopotentiating effect of fungal glucans as revealed by frequency limitation of post-chemotherapy relapse in experimental mouse tuberculosis. Jpn J Med Sci Biol 1980; 33:283–93.
9. Byram JE, Sher A, DiPietro J, von Lichtenberg F. Potentiation of schistosome granuloma formation by lentinan—A T-cell adjuvant. Am J Pathol 1979; 94:201–18.
10. Maeda YY, Chihara G. Lentinan, a new immunoaccelerator of cell-mediated responses. Nature 1971; 229:634.
11. Maeda Y, Chihara G. The effect of neonatal thymectomy on the antitumor activity of lentinan, carboxymethylpachymaran and zymosan, and their effects on various immune responses. Int J Cancer 1973; 11:153–61.
12. Dennert G, Tacker D. Antitumor polysaccharide lentinan—A T-cell adjuvant. JNCI 1973; 51:1727–9.
13. Dresser DW, Phillips JM. The orientation of the adjuvant activities of *Salmonella typhosa* lipopolysaccharide and lentinan. Immunology 1974; 27:895–902.
14. Haba S, Hamaoka T, Takatsu K, Kitagawa M. Selective suppression of T-cell activity in tumor bearing mice and its improvement by lentinan, a potent antitumor polysaccharide. Int J Cancer 1976; 18:93–104.
15. Hamuro J, Wagner H, Röllinghoff M. β-1,3-Glucans as probe for T-cell specific immune adjuvants. Enhanced in vitro generation of cytotoxic T-lymphocytes. Cell Immunol 1978; 38:328–35.
16. Hamuro J, Röllinghoff M, Wagner H. β-1,3-Glucans mediated augmentation of alloreactive murine cytotoxic T-lymphocytes in vivo. Cancer Res 1978; 38:3080–5.
17. Hamuro J, Röllinghoff M, Wagner H. Induction of cytotoxic peritoneal exudate cells by T-cell immune adjuvants of β-1,3-glucan type lentinan and its analogues. Immunology 1980; 39:551–9.
18. Hamuro J, Chihara G. Lentinan, a T-cell oriented immunopotentiator: Its experimental and clinical applications and possible mechanism of immune modulation. In Fenichel RL, Chirigos MA, eds: Immune modulation agents and their mechanisms. New York: Marcel Dekker, 1985: 409–36.
19. Sortwell RJ, Dawe S, Allen DG, et al. Chronic intravenous administration of lentinan to the rhesus monkey. Toxicol Lett 1981; 9:81–5.
20. Taguchi T, Furue H, Kimura T, Kondo T, Hattori T, Ogawa N. Clinical efficacy of lentinan on neoplastic disease. In Klein K, Spector S, Friedman H, Szentivanyi A, eds: Biological response modifiers in human oncology and immunology. New York: Plenum Press, 1983; 181–7.
21. Taguchi T, Furue H, Kimura T, et al. End-point results of phase III study of lentinan. Gan to Kagakuryoho [Jpn J Cancer Chemother] 1985; 12:366–78.
22. Klein G, Sjögren O, Klein E, Hellström KE. Demonstration of resistance against methylcholanthrene-induced sarcoma in the primary autochthonous host. Cancer Res 1960; 20:1561–72.
23. Watanabe H, Suemasu K. Cancer surgical therapy and lymph node metastases. Oncologia 1982; 1:83–9.
24. Rose WC, Reed III FC, Siminoff P, Bradner WT. Immunotherapy of Madison 109 lung carcinoma and other murine cancer using lentinan. Cancer Res 1984; 44:1368–73.
25. Maeda YY, Hamuro J, Chihara G. The mechanisms of action of antitumor polysaccharide: The effect of antilymphocyte serum on the antitumor activity of lentinan. Int J Cancer 1971; 8:41–6.
26. Izawa M, Ohno K, Amikura K, Hamuro J. Lentinan augments the production of Interleukin 3 and colony stimulating factor(s) by T-cells. In Aoki T, Tsubura E, Urushizaki I, eds: Manipulation of host defence mechanisms. Amsterdam: Excerpta Medica, 1984; 59–69.
27. Maeda YY, Watanabe ST, Chihara G, Rokutanda M. T-cell mediated vascular dilatation and hemorrhage induced by antitumor polysaccharides. Int J Immunopharmacol 1984; 5:493–501.
28. Suga T, Maeda YY, Uchida H, Rokutanda M, Chihara G. Macrophage-mediated acute-phase transport protein production induced by lentinan. Int J Immunopharmacol 1986; 8:691–9.
29. Maeda YY, Chihara G, Ishimura K. Unique increase of serum proteins and action of antitumor polysaccharides. Nature 1974; 252:250–2.
30. Manabe T, Takahashi Y, Okuyama T, Maeda YY, Chihara G. Identification of mouse serum proteins increased by the antitumor polysaccharide lentinan by micro two dimensional electrophoresis. Electrophoreses 1983; 4:242–6.
31. Fruehauf JP, Bonnard GD, Herberman RB. The effect of lentinan on production of Interleukin-1 by human monocytes. Immunopharmacology 1982; 5:65–74.

32. Zákány J, Chihara G, Fachet J. Effect of lentinan on the production of migration inhibitory factor induced by syngeneic tumor in mice. Int J Cancer 1980; 26:783–8.
33. Spika S, Ábel G, Csongor J, Chihara G, Fachet J. Effect of lentinan on the chemiluminescence produced by human neutrophils and the murine macrophage cell line C4MØ. Int J Immunopharmacol 1985; 7:745–51.
34. Okuda T, Yoshioka Y, Ikekawa T, Chihara G, Nishioka K. Anticomplementary activity of antitumor polysaccharides. Nature [New Biol] 1972; 238:59–60.
35. Hamuro J, Hadding U, Bitter-Seuermann D. Solid phase activation of alternative pathway of complement by β-1,3-glucans and its possible role for tumor regressing activity. Immunology 1978; 34:695–705.
36. Maeda YY, Chihara G. Periodical consideration on the establishment of antitumor action in host and activation of peritoneal exudate cells by lentinan. Gann [Jpn J Cancer Res] 1973; 64:351–7.